Expression and function of Leukotriene B4 receptors in human articular chondrocytes

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/ ABSTRACT

Leukotriene B4 (LTB4) is linked to osteoarthritis (OA) development however the expression of LTB4 receptors in cartilage cells and the physiological effects of LTB4 on cartilage tissue remain unknown. In this study we find that human articular chondrocytes express LTB4 receptors and that these receptors are functional, however, LTB4 does not seem to affect importantly some primary chondrocyte functions.

/ PURPOSE

Leukotriene B4 (LTB4) is an arachidonic acid metabolite that exerts potent leukocyte chemotaxis and other pro-inflammatory reactions. Previous studies have demonstrated that LTB4 has a role in development of RA and OA by acting on G-coupled receptors leukotriene B4-receptor 1 and 2 (BLT1 and BLT2). Activation of BTL1 and BLT2 is confirmed in leukocytes and synovial tissue, but proof of their presence in articular cartilage is lacking. In this study we aim at clarifying whether cartilage cells express BLT1 and BLT2 and we explore the potential effects elicited by LTB4 on chondrocyte behaviour.

/ METHODS AND MATERIALS

- Human material and cell cultures: Cartilage tissue harvested from macroscopically healthy areas of distal femurs during knee replacements (N=8, age=55-72). Cultures chondrocytes represent surplus cells from ACI operations at passage 3 or 4 (N=7, age=30-42).
- Immunohistochemistry and immunocytochemistry: Cells and tissues fixed with formalin, permeabilized and stained with specific polyclonal Rabbit anti BLT-1 and BLT-2 antibodies.
- qPCR: predesigned TaqMan probes targeting cartilage signature genes (Aggrecan, Collagen type I, Collagen Type II, Sox-9).
- Receptor activation and Inhibition: ERK ½ phosphorylation analysed by western blot. BLT1 and BLT2 receptor blockage using excess amounts of receptor antagonists U-75302 and LY2552833 respectively.
- Multiplex protein assays: Bead-based Multi Analytes Profiling Assays (LUMINEX-Bio-Rad):
  - MMP kit assay: MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13
  - Inflammatory cytokine assay: IL-1β, TNF-α, IL-6, IL-8, MCP-1
  - Growth factors: VEGF, bFGF and PDGF
- 3D in vitro assays: scaffold-free chondrocytes in pellets (50.000 cells/pellet) generated during 48 hours and then incubated for 1 week in serum-free medium containing LTB4.
- Histology: Alcian blue for identification of proteoglycans and collagen type II for detection of cartilage specific collagen type II.

/ RESULTS

/ EXPRESSION OF BLT1 AND BLT2 IN NATIVE CARTILAGE

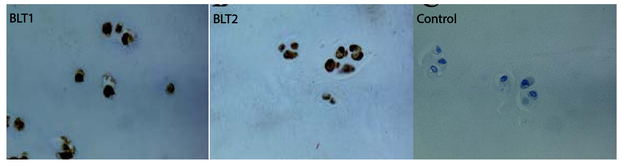

Figure 1: Immunohistochemistry (60x micrograph) Cartilage tissue sections stained with BLT1 antibody, BLT2 antibody and isotype control. Brown staining indicate binding of antibody to BLT1 and BLT2 respectively.

/ EXPRESSION OF BLT1 AND BLT2 IN CULTURED CHONDROCYTES

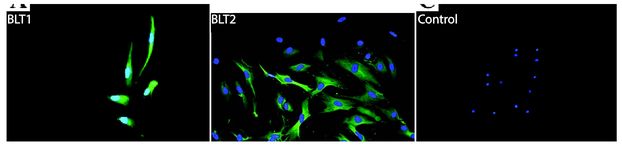

Figure 2: Immunocytochemistry (60x micrograph). Fluorescence microscopy of cultured chondrocytes stained with BLT1 antibody, BLT2 antibody and no antibody (control). Secondary antibody conjugated with Alexa Fluor488 and DAPI stained nuclei. Green staining indicate binding of antibody to BLT1 and BLT2 respectively.

/ MRNA EXPRESSION OF BLT1 AND BLT2 IN CHONDROCYTES

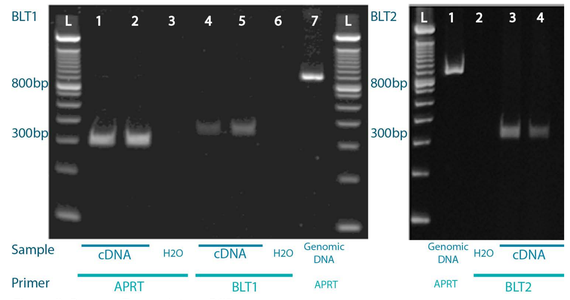

Figure 3. Reverse Transcriptase PCR
BLT1 Lane L: DNA ladder, Lane 1 and 2: mRNA control, purity of cDNA is confirmed by 300bp bands and no trace of the 800bp band that appears when APRT is primed to genomic DNA as seen in lane 7. Lane 3 and 6: Negative control. Lanes 4 and 5 shows that specific BLT1-primers and cDNA from two different cell cultures give 345 bp bands, indicating transcript for the BLT1 receptor.
BLT2 Lane L: DNA ladder. Lane 1: mRNA control. Lane 2: Negative control. Lanes 3 and 4 shows that specific BLT2-primers and cDNA from two different cell cultures give two 320 bp bands, indicating transcript for the BLT2 receptor.

/ ACTIVATION OF G-COUPLED RECEPTOR BLT1 – WESTERN BLOTTING OF DOWNSTREAM PHOSPHORYLATION OF ERK1/2

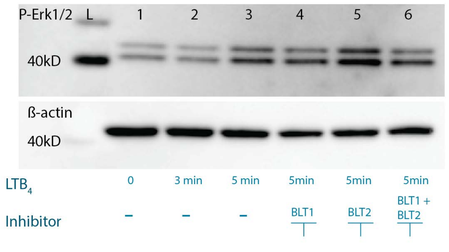

Figure 4. P-Erk1/2 (44/42 kD).
Lane 1-3 shows that Erk1/2 phosphorylation is strongest at 5 minutes exposure to LTB4. Lane 4 and 6 indicates reduced phosphorylation of Erk1/2 when BLT1 is blocked. Blocking of BLT2 does not seem to have the same effect (Lane 5).
Beta-actin (45kD) is the loading control.

/ BIOLOGICAL FUNCTIONS: REGULATION OF CYTOKINES, GROWTH FACTORS AND MMPS

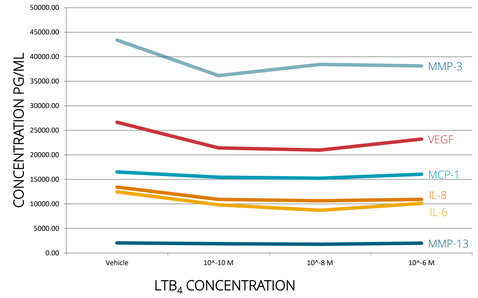

Figure 5. Multiplex protein assays. Levels measured in supernatants after challenging cultured chondrocytes for 24 hours with LTB4 at concentrations of 10^{-10}M, 10^{-8}M, 10^{-6}M and vehicle. Only detectable molecules are represented in the graphics. We found no significant variations in released factors analysed after LTB4 exposure.

/ BIOLOGICAL FUNCTIONS: REGULATION OF CARTILAGE SIGNATURE GENES

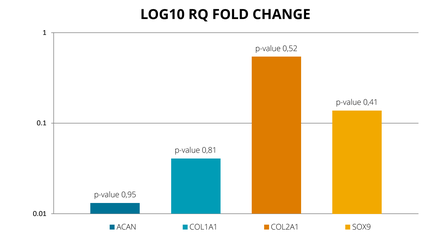

Figure 6. Relative expression of cartilage signature genes in LTB4 challenged chondrocytes versus untreated chondrocytes. Results show no significant differences in the transcription of standard cartilage marker genes

/ BIOLOGICAL FUNCTIONS: REGULATION OF CARTILAGE FORMATION IN 3D IN VITRO MODEL

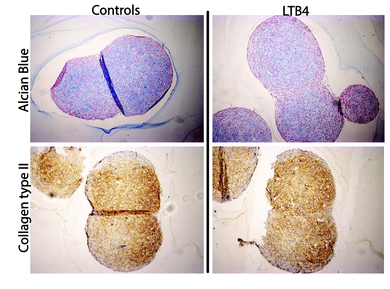

Figure 7. Histology and immunohistochemistry of scaffold-free chondrocyte spheroids incubated for 7 days in LTB4 at a concentration of 10^{-7}M vs. unchallenged controls. Brown colour corresponds to expression of collagen type II, whereas blue colour is indicative of matrix proteoglycans. No marked differences on cartilage matrix components are observed after LTB4 exposure.

/ CONCLUSION

Chondrocytes express functional BLT1 and BLT2 receptors and we find proof of downstream activation of high affinity receptor BLT1. Central cell functions such as proliferation, cartilage gene expression, matrix formation or expression of other inflammatory molecules were unaffected by LTB4. Overall the biological significance of LTB4 on cartilage physiology still needs to be determined.

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